Faculty of Science

EGG COLOURATION IN MOUNTAIN BLUEBIRDS (*SIALIA CURRUCOIDES*): A SEXUALLY SELECTED INDICATOR OF FEMALE QUALITY?

2015 | JACOB M. BAILEY

B.Sc. Honours thesis

THOMPSON RIVERS UNIVERSITY

EGG COLOURATION IN MOUNTAIN BLUEBIRDS (*SIALIA CURRUCOIDES*): A SEXUALLY SELECTED INDICATOR OF FEMALE QUALITY?

by

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
BACHELOR OF SCIENCE (HONS.)
in the
DEPARTMENT OF BIOLOGICAL SCIENCES
ECOLOGY AND ENVIRONMENTAL BIOLOGY

THOMPSON RIVERS UNIVERSITY

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Dated this 27th day of April, 2015, in Kamloops, British Columbia, Canada

ABSTRACT

An adaptive explanation for the blue-green colouration of some avian eggshells has long interested researchers and naturalists alike. Unlike brown and maculated eggs that probably evolved through selection for crypsis, blue-green eggs would likely appear conspicuous to predators. It has been proposed that blue green egg colouration may be a sexually selected indicator of female quality. Because the pigment responsible for blue egg colouration is also an antioxidant, egg colour may act as a post-mating signal to the male of the laying female's quality. Males may thus use egg colour to assess the female's quality, and therefore, the quality of her offspring, investing more heavily in clutches of better quality females. The mountain bluebird (*Sialia currucoides*) is a socially monogamous species with biparental care, which lays blue eggs, making it an excellent species to test the sexual selection hypothesis for the evolution of blue egg colouration. Using digital photography, I examined the blue colouration of mountain bluebird eggs to investigate evidence for blue egg colouration acting as a sexually selected signal in mountain bluebirds. There were no consistent relationships between egg colouration and other variables, including parental provisioning rates, female condition, or offspring survival and growth rates. I conclude that there is insufficient evidence to support the notion that mountain bluebirds are using egg colouration as a sexually selected signal. However, variation in egg blue coloration was related to female provisioning rates, suggesting there may be a link between female quality and egg colouration.

Thesis Supervisor: Assistant Professor, Dr. Matthew Reudink

ACKNOWLEDGEMENTS

I would like to thank Dr. Reudink for being such a phenomenal supervisor and mentor, but most of all for sharing his passion for ornithology with me. This project has been an invaluable part of my university experience, one that I would have likely missed out on had it not been for him. I would like to thank all the members of our lab, especially Sarah McArthur, for their technical and intellectual assistance both in the field and lab. Special thanks to Stephen Joly for sharing his expertise, and his help catching and banding bluebirds. Thanks to the Kamloops Naturalist Club for their help and cooperation during this project and in ongoing research, and to the Natural Sciences and Engineering Research Council of Canada for funding this project. And finally, thank you to my parents, Brian and Donna, and to my siblings, Emily and Noah, for supporting me.

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INTRODUCTION

Avian eggshells show great diversity in their colouration and patterning both across and within species, with colouration ranging from white to brown, to green, to blue. Eggs may be spotless, densely speckled, or somewhere in between (Kennedy & Vevers 1976; Cassey et al. 2010). Many hypotheses have been put forth to explain the evolution of this vast diversity in appearance. Egg colouration may have evolved in response to selection for crypsis (Lack 1958), mimicry of host eggs by brood parasites or brood parasite recognition by hosts (Newton et al. 1896), filtering of solar radiation (Montevecchi 1976), or potentially as a sexually selected signal (Moreno and Osorno 2003; Moreno et al. 2005, 2006; Soler et al. 2005; Hanley et al. 2008; Walters and Getty 2010; English and Montgomerie 2011).

Although theories explaining the evolution of egg coloration are diverse and often species specific, the mechanisms controlling egg colour are better understood and more precise. Egg colour is produced by pigments formed from the catabolism of haemoproteins deposited in the shell (Battersby et al. 1980; Kutty & Maines 1984). After release from the ovary, the fertilized ovum moves to the shell gland pouch, where biomineralization occurs; here, the majority of the shell is laid down, along with the pigments responsible for the colour and patterning of the shell (Board & Sparks 1991; Gosler et al. 2005). These pigments are only deposited shortly before egg laying and are therefore part of the outermost shell and cuticle (Burley & Vadehra 1989; Soh et al. 1993). Eggshell colouration in birds is attributed to two main pigments: brown and reddish colouration is produced by deposition of the pigment protoporphyrin, whereas blue-green colouration results from deposition of the pigment biliverdin (Kennedy & Vevers 1976; Burley & Vadehra 1989; Mikšík et al. 1996). Simultaneous expression of both protoporphyrin and biliverdin results in an array of olive

colours, depending on the relative expression of the two pigments (Collias 1993). The amount of these pigments deposited in the eggshell is thought to be controlled by the female's estradiol and progesterone levels during egg laying (Soh & Koga 1997).

Interestingly, while the eggs of some species appear completely white and free of any speckling, they can contain both protoporphyrin and biliverdin. The presence of these pigments even in the absence egg colouration suggests they may also serve a structural function, contributing to the strength and flexibility of the eggshell, which may be especially important for smaller passerines (Kennedy & Vevers 1976; Miksik et al. 1994; Gosler et al. 2005). Because small passerines have a smaller skeleton-to-egg size ratio than larger passerines, they should have relatively limited amounts of calcium available for deposition in eggshells and may thus need to compensate with additional structural elements. For instance, great tits (*Parus major*) increase deposition of the pigments protoporphyrin and biliverdin in response to eggshell thinning, indicating that deposition of greater amounts of pigment may be a compensatory mechanism to maintain eggshell strength and integrity (Gosler et al. 2005). These findings suggest that protoporphyrin and biliverdin may have been of structural importance ancestrally, but have since been adapted to serve such functions as signaling and crypsis (Kilner 2006).

One of the more well-supported theories for the evolution of brown and maculated eggs, from a presumably white ancestral state (Kilner 2006), is that this is adaption provides crypsis. Supporting this theory, brown and maculated eggs are especially common in species that nest in exposed sites as compared to cavity nesters (Lack 1958; Westmoreland & Kiltie 2007; Westmoreland 2008). Skrade & Dinsmore (2013) used digital photography to investigate the predation rates of mountain plover (*Charadrius montanus*) eggs with respect

to their level of crypis. Nest survival rates increased as the difference in colouration between eggs and nest substrate decreased, demonstrating that more cryptic eggs had a higher probability of survival in a ground nesting species with maculated eggs. The same relationship was demonstrated in black-tailed gulls (*Larus crassirostris*), with colour matching between substrate and egg colour predicting the likelihood that a nest would survive to hatching (Lee et al. 2010).

Unlike the situation for brown and maculated eggshells, an adaptive explanation of blue-green egg colouration remains somewhat more elusive (Cherry & Gosler 2010). If blue-green egg colouration is as conspicuous to predators as it is to humans, cryptic function does not provide an adequate evolutionary explanation for blue-green egg colouration. One possibility is that the intensity of blue egg colouration may be acting as a sexually selected indicator of female quality. Moreno & Osorno (2003) proposed that because males have numerous opportunities to visit the nest during the laying and incubation period, a male may use these observational visits to evaluate his mate's quality through the colour of her eggs. According to the differential allocation hypothesis, in species with biparental care, offspring of high quality mates would warrant greater parental investment than those of lesser a quality mate (Burley 1986). By assessing the quality of the laying female post-mating, males may be better positioned to determine and adjust their level of subsequent parental investment.

To be a reliable signal of female quality, egg colour should incur a cost to the laying female (Zahavi 1975). By being costly to produce, a signal honestly conveys the individual's quality because an individual of lower physiological or genetic quality could not afford the same trait. Indeed, biliverdin, the pigment responsible for the blue colouration of avian eggshells, appears to be costly for laying females to produce. Thus, eggshell pigmentation

may function as a signal of quality, analogous to the well-studied situation for other antioxidant pigments, such as carotenoids, which are deposited in plumage. Carotenoid-based plumage and integumentary colouration has been repeatedly implicated as an honest indicator of individual condition and quality and often acts an important signal in intraspecific interactions (Hill & Montgomerie 1994; Horak et al. 2000; Senar et al. 2003; Germain et al. 2010). Similarly, biliverdin, which is formed from the breakdown of haem in the liver (Bauer & Bauer 2002), has been shown to have potent antioxidant function against dangerous peroxyl and hydroxyl free radicals, such as hydrogen peroxide and nitric oxide (McDonagh 2001; Kaur et al. 2003; Jansen et al. 2010). Given the handicap of antioxidant removal from use in the female's immune function, biliverdin deposition into eggshells may signal the laying female's capacity to handle free radicals despite this. Furthermore, it has been demonstrated that steroid hormones can negatively impact enzymatic antioxidant defenses in addition to inducing oxidative stress (von Schantz et al. 1999). Therefore, biliverdin eggshell deposition may not only be signaling laying female antioxidant capacity, but doing so during an energetically stressful time, thus compounding the antioxidant handicap effect of eggshell pigment deposition.

In order for evolution to act on a trait, there must be some heritable genetic component to the phenotypic variation of the trait. In poultry, two autosomal loci have been demonstrated to affect eggshell colouration (Wei et al. 1992). For example, in Japanese quail (*Coturnix japonica*) a mutation controlled by an autosomal recessive gene reduces the content of both biliverdin and protoporphyrin deposited in the eggshell and has been shown to have a significant effect on eggshell colouration (Ito et al. 1993). A five-year study of pied flycatchers (*Ficedula hypoleuca*) demonstrated that heritability of egg colouration was

moderately high for all measured colour variables, with heritability explaining between 15% to 54% of the total phenotypic variation in egg colour (Morales et al. 2010a). Within clutch variation in egg colour had the highest coefficient of additive genetic variation and therefore had the highest potential for evolutionary change.

One of the vital facets of the sexual selection hypothesis is the assumption that offspring hatching from bluer eggs are of higher quality, and therefore warrant greater paternal investment. By measuring yolk levels of maternally derived antibodies, (Morales et al. 2006) demonstrated a positive relationship between egg blue colouration and immunocompetence. Maternally derived antibodies are important for the avian humoral immune response early in life, and are associated with offspring survival (Starck & Ricklefs 1998). The link between egg colouration and egg quality thus provides a basis for the assumption that egg blue colouration may be acting as a signal of higher quality in offspring.

In the same study, Morales et al. (2006) demonstrated a relationship between female quality and egg blue colouration. Females of better quality laid clutches that were, on average, bluer than those of lower quality females. This relationship has also been shown to exist in eastern bluebirds (*Sialia sialis*), with females in better physiological condition laying bluer eggs on average (Siefferman et al. 2005). Researchers have also been able to experimentally increase the amount of blue in the eggs of a clutch through supplementation of female diet during the laying period (Moreno et al. 2006a). Females supplemented with mealworms, laid eggs that were significantly bluer, with higher levels of eggshell biliverdin, compared to those laid by control females. More recently, work with pied flycatchers has provided additional support for the relationship between female antioxidant capacity and egg clutch blue colouration (Moreno et al. 2013). By taking blood samples of laying females and

quantifying egg colouration, Moreno et al. (2013) demonstrated a positive correlation between antioxidant levels in females and the blue colouration of their eggs. Similarly, female gray catbirds (*Dumetella carolinensis*) with higher antioxidant capacity laid bluer eggs, providing yet another potential link between female condition and egg colouration (Hanley et al. 2008). Soler et al. (2008) demonstrated that by removing feathers to manipulate female body condition in spotless starlings (*Sturnus unicolor*), they were able to decrease the level of blue colouration in clutches, while diet supplementation during the egg laying period increased egg antibody levels and the blueness of eggs. Together these results show strong support for a link between female condition and egg colouration, and thus support for the sexual selection hypothesis to explain egg colouration, in at least these species

The next crucial aspect of the sexual selection hypothesis to investigate is if males adjust their parental investment in response to perceived offspring quality, as signaled by eggshell colouration. There is both experimental and observational evidence to support this claim. Observationally, in pied flycatchers and grey catbirds, male parents of clutches with bluer eggs, show greater parental investment during the nestling stage (Soler et al. 2008; Hanley et al. 2008). Researchers have also found experimental evidence that male birds adjust their parental investment in response to the amount of blue colouration in eggs of the spotless starling and American robin (*Turdus migratorius*) (Soler et al. 2008; English & Montgomerie 2011). In both studies, researchers replaced real eggs with artificial ones of varying blue colouration, then added cross fostered nestlings to measure parental investment in terms of provisioning rates. In both instances males at nests with bluer eggs had higher provisioning rates.

In this study, I investigate evidence for the sexual selection hypothesis as an explanation for the blue colouration of mountain bluebird (*Sialia currucoides*) eggs. Mountain bluebirds are a cavity nesting species that exhibits biparental care, has colourful ornamental plumage that appears to be sexually selected (Balenger et al. 2007; Morrison et al. 2014), and has bright blue eggs, thus making them an excellent species with which to study the sexual selection hypothesis for evolution of bright egg colouration. Furthermore, studies on two other species in the Turdidae family (American robin and eastern bluebird) have provided evidence to support the sexual selection hypothesis. Specifically, in this study I ask (a) if female condition was positively correlated with blue egg colouration, (b) if males increase their parental investment in terms of provisioning rates in response to variation in blue colouration of eggs, and (c) if offspring hatched from bluer eggs grew faster or had higher fledging success.

MATERIALS AND METHODS

Field methods

Field work was conducted during the during the 2014 breeding season (May–July) on a population of mountain bluebirds inhabiting 77 nest boxes, near Kamloops, BC, Canada (50°37’ N, 120°19’W; elevation: 885–1116 m;). Each nest box was monitored every 1-3 days to determine first egg date, number of eggs, hatch date, number of nestlings, and fledging success (empty nests on or after the predicted date of fledging were considered to have successfully fledged; the number of fledglings was estimated as the most recent number of nestlings recorded prior to fledging). Adult females (n=18) and males (n=16) were captured using box traps and banded with a Canadian Wildlife Service aluminum band and PIT tag

(for a concurrent study) 5-10 days after hatch date. Birds were sexed based on plumage colouration and classified as either second-year (SY) or after-second-year (ASY) by examining moult limits of the primary and greater coverts (Pyle 1997). I collected feather samples from both the rump and tail (R3) of each captured adult bird. I also recorded the mass, unflattened wing chord, tail length, and tarsus length for each bird. Nestling wing length, mass, and tarsus length were measured on days 5, 9, and 13 after hatching. Nestlings were banded with a single Canadian Wildlife Service aluminum band.

Parental care

I quantified parental care by video recording provisioning trips during two-hour periods during the early (3–5 d after hatching) and late (14–16 d after hatching) nestling phases for a total of 4h per nest, following Balenger et al. (2007). Nest watch videos were recorded using either a Handycam DCR-SX45 (Sony, Tokyo, Japan) or a HD Hero2/3 (GoPro, San Mateo, CA). All nest watches occurred between 7:57 AM and 12:45 PM (mean = 10:07AM ± 66 min). Video cameras were placed on the ground ~2m in front of the nest box, facing upwards at the box entrance. Provisioning rate was measured as the number of trips to the nest per hour per nestling. Female visits to the nest lasting >30 s during the early nestling phases were recorded as brooding. I also recorded the number of nestlings present in each nest during the watch periods. I did not capture adults at nests within the 48 h preceding measurement of provisioning rates.

Egg colouration analysis

I used digital photography and Photoshop CS6, version 13.0 (Adobe Systems Incorporated, San Jose, California 2012) for egg colour analysis. Methods were adapted from McCormack and Berg (2010) and Negro (1998). Because birds can see into the UV light spectrum, birds

may be using different colour cues that the ones apparent to the human eye in order to assess egg colouration (Bennett & Cuthill 1994). While digital photography is not able to measure colouration into the UV spectrum (300-400 nm), the maximal reflectance of the eggs of other species with similarly coloured eggs (including the closely related eastern bluebird), falls within the human visible range at approximately 500nm, and is likely correlated with spectra in the UV range (Morales et al. 2010a). I took various steps to address potential difficulties associated with using digital photography to quantify colour (Stevens et al. 2007; McCormack and Berg 2010). (1) All photos were taken with the same camera (Panasonic Lumix DMC-FZ8) with the same settings in manual mode. (2) I used a background standard with each photo to correct for ambient light variation among photos. (3) I took precautions to avoid over and under-exposure by photographing eggs away from direct sunlight in nest boxes and by photographing eggs from a consistent distance.

I used JPG image file formats to analyze the photos in Photoshop. JPG file types may result in some loss of detail, as compared to higher resolution file types such as TIFF/RAW files; however, because I was interested in eggshell pigment colouration that is deposited uniformly over the eggs' surface, detail was of less concern. To calculate the mean colour values for each egg, I used the “marquee” tool and the histogram interface in Photoshop to select the egg to be analyzed, avoiding areas of high reflectance or obstruction. The “marquee” tool was also used to obtain the mean RGB values from the colour standard in each photo. To calculate the corrected blue (B) value for each egg, the blue value from the black portion of the standard in every photo taken and averaged. This average was then subtracted from the blue value of the black portion of the standard in each clutch photo to yield either a positive or negative correction factor specific to each clutch, depending on if

the photo was either over or under saturated. The standard deviation of the blue value for the eggs in a single clutch was calculated using these corrected values.

Plumage colouration analysis

I used reflectance spectrometry with a xenon light source (JAZ, Ocean Optics Dunedin, FL) to quantify adult bird plumage colouration across the avian visual spectrum (300-700 nm), following Morrison et al. (2014). I mounted feathers on low reflectivity black paper (Ebony #142, Canson, Inc., South Hadley, MA); rump feathers were mounted in an overlapping pattern. A fiber optic probe was held in a non-reflective holder at a 90° angle, 5.9 mm from the feather surface. I took 10 measurements from both the tail and rump feathers at haphazard regions along their lengths. Using the R-based colour analysis program RCLR v.28 (Montgomerie 2008), I calculated three discrete variables (brightness, UV-blue chroma, and hue) and obtained the average value for each variable over the ten measurements. Brightness, the amount of light reflected by the feather, was calculated as the percent reflectance averaged across the visual spectrum (300-700 nm). Chroma was calculated as the fraction of total light reflected (300-700nm) that was within both the blue spectrum (400-510 nm) and the ultraviolet spectrum (UV) (300-400 nm), and represents the saturation or spectral purity. Hue was calculated as the wavelength of maximum reflectance. Hue gives information on the dominant wavelength of light reflected, i.e., the colour the feather appears. Because there was a high degree of colinearity between brightness, UV chroma, and hue, I combined the three variables into one using principal component analysis in JMP 11 (SAS institute 2013), following Morrison et al. (2014). The first principal component (PC1) explained most of the variation for each plumage area (Table 1). Therefore, I used variation

in PC1 to represent overall colour variation. PC1 scores loaded positively on brightness and UV-blue chroma, and negatively on hue (lower hue values indicate a shift toward UV) (Table 1).

Table 1. Results from principle components analysis of measures of plumage colouration (brightness, hue, and chroma) for rump and tail colouration of male and female Mountain Bluebirds. Because the first principle component (PC1) explained most of the variation, I used PC1 to represent overall colour variation.

	Eigenvalue	Proportion of variance	Colour variable	Factor loading
Male tail PC1	1.88	0.63	Brightness	0.61
			UV + blue choma	−0.60
			Hue	0.52
Female tail PC1	1.77	0.59	Brightness	0.54
			UV + blue choma	−0.64
			Hue	0.55
Male rump PC1	2.2	0.73	Brightness	0.50
			UV + blue choma	−0.59
			Hue	0.42
Female Rump PC1	2.09	0.7	Brightness	−0.64
			UV + blue choma	0.65
			Hue	

Statistical analysis

Statistical analysis was performed using JMP 11 (SAS institute 2013). All analyses were performed separately for males and females. As my data fit the assumptions of normality, I used Pearson's correlations to determine relationships between egg colour, provisioning rates, and nestling condition and fledging success. T-tests were used to examine whether there were differences in average egg colour between clutches that fledged and those that didn't. Differences in sample sizes between early and late stages were the result of nest failure between stages. Similarly, differences in male and female sample size were due either to a failure to capture individual adult birds or possible mortality.

RESULTS

Provisioning rates and egg colouration

Contrary to my prediction, there was no relationship between early nestling stage provisioning rates and the average egg blue colouration in either males or females (males: $n = 19$, $r = 0.04$, $p = 0.88$; females: $n = 18$, $r = -0.26$, $p = 0.30$, figure 1A). However, when one outlier (defined as any data point falling outside of 1.5 inter quartile ranges below the first quartile or above the third quartile) was excluded from the analysis there was a significant negative correlation between the early nestling stage provisioning rates of females and the average blue colouration of their clutches ($n = 17$, $r = -0.53$, $p = 0.03$, figure 1B), that is

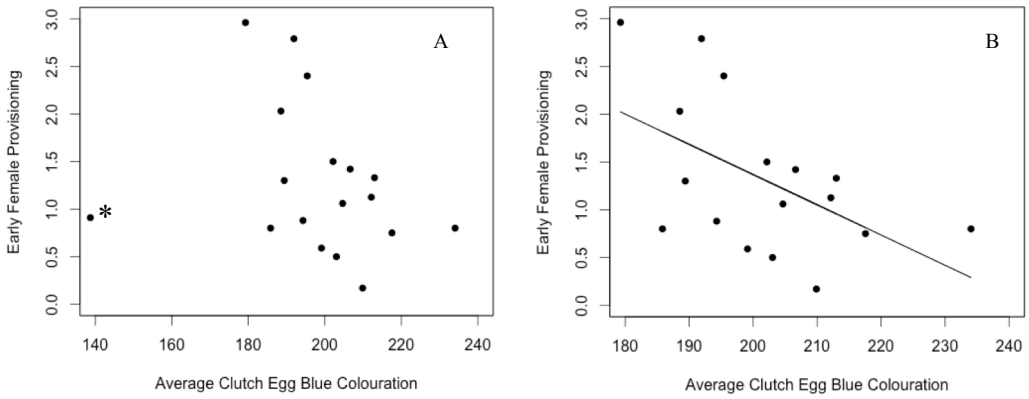

Figure 1. There was no relationship between early female provisioning trips and average clutch egg blue colouration females (A). When the outlier (denoted by *) was excluded from the analysis it revealed a negative relationship between early female provisioning and average clutch egg colouration (B).

clutches with bluer eggs were provisioned at lower rates.

I found no relationship between late nestling stage provisioning rates and average egg blue colouration in either males or females (males: $n = 17$, $r = -0.53$, $p = 0.76$ figure 2A; females; $n = 17$, $r = -0.13$, $p = 0.62$). When the same outlier clutch was excluded from the

analysis there was a marginally negative relationship between late nestling stage male provisioning rates and average egg blue colouration ($n = 17$, $r = -0.49$, $p = 0.052$, figure 2B). There was also no relationship between total (combined male and female) nestling provisioning rates and average clutch egg blue colouration during either the late or early nestling stage (early nestling stage: $n = 19$, $r = -0.20$, $p = 0.40$; late nestling stage: $n = 17$, $r = -0.26$, $p = 0.31$, figure 3A). Again, when the same outlier clutch was excluded, there was a negative relationship between total late nestling stage provisioning rates and average clutch egg blue colouration ($n = 16$, $r = -0.52$, $p = 0.04$, figure 3B).

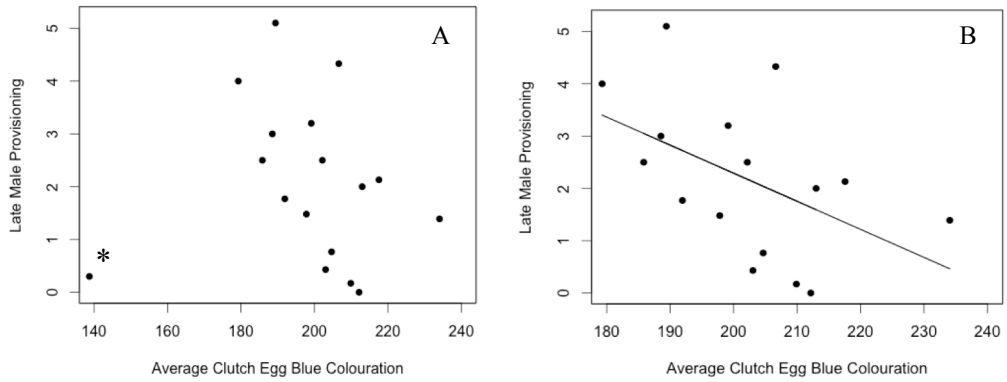

Figure 2. There was no relationship between late male provisioning trips and average egg blue colouration (A). When the outlier (denoted by *) was omitted from analysis there was a marginal negative relationship between late male provisioning and average egg blue colouration (B).

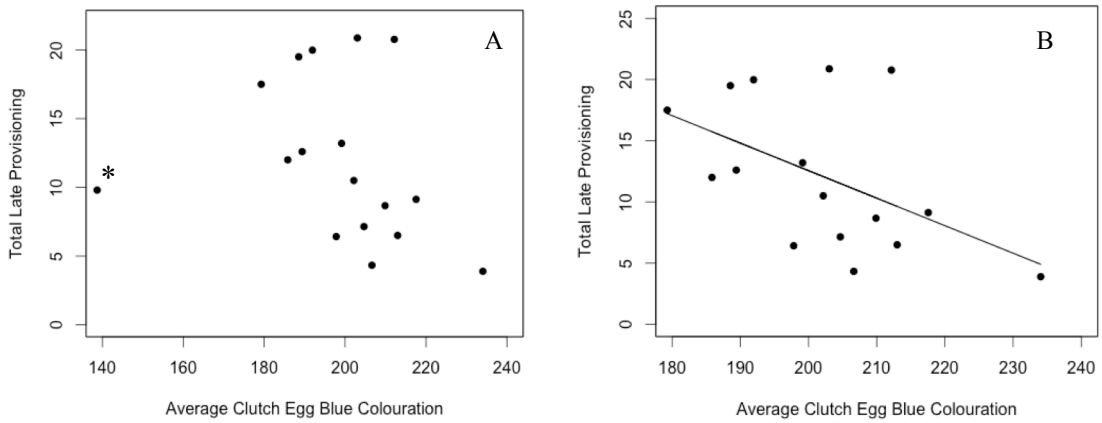

Figure 3. There was no relationship between total late provisioning trips and average egg blue colouration (A). When the outlier (denoted by *) was excluded from analysis there was a negative relationship between total late provisioning and average egg blue colouration (B).

I also investigated whether variation in egg colouration within a nest predicted provisioning rates and found that clutches with greater variation (i.e., measured as the standard deviation of the blueness of the eggs in a nest) in blue colouration within a clutch received higher maternal investment during the early nestling stage (n = 18, r = 0.54, p = 0.02, figure 4).

Though there is evidence to suggest that blueness decreases with each subsequent egglaid (Moreno et al. 2005), I found no relationship between the colouration of the bluest egg in the clutch (presumably the first laid egg) and provisioning rates during the early (males, n = 19, r = −0.14, p =0.56; females, n = 18, r = −0.05, p = 0.84) or late (males, n = 17, r = 0.02, p = 0.93; females, n = 17, r = −0.10, p = 0.70) nestling stage.

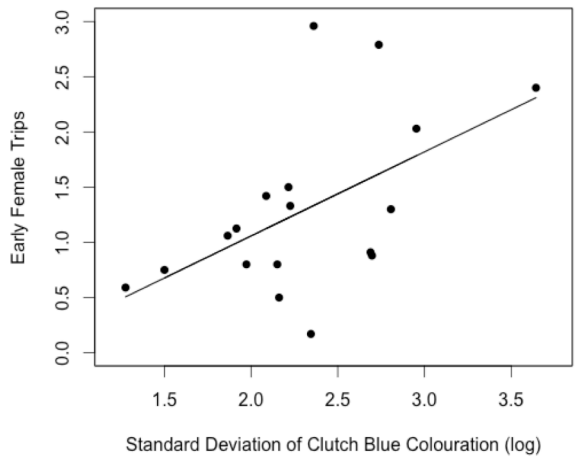

Figure 4. Early nestling stage female provisioning rates were positively correlated with increased variation of blue colour within a clutch.

Female plumage colour and egg colouration

I found no relationship between the rump PC1 of females and the average blue colouration of the eggs in their nests (n = 18, r = −0.01, p = 0.97); the same was true for female tail PC1 and average egg blue colouration (n = 18, r = −0.25, p = 0.32).

Fledging and egg colouration

Clutches of greater average blue colouration were not more likely to fledge successfully, nor did clutches of greater average egg blue colouration grow faster, hatch sooner, or fledge sooner. There was no relationship between average nestling growth rates and average clutch blue colouration (p > 0.74 for all tests, see Appendix 1A). Blue colouration did not differ between clutches that fledged and those that did not (n = 17 fledged, n = 9 did not fledge, t = −0.30, p = 0.77, figure 5). Average blue colour of clutches that fledged was 199 (± 20.36), while the average blue colour of those that did not was 201 (± 15.89) (RGB scale).

There was no relationship between hatch date (Julian date from May 1st) and average egg blue colouration (n = 20, r = 0.17, p = 0.47). Likewise, fledge date was not correlated with average egg blue colouration (n = 16, r = 0.76, p = 0.58).

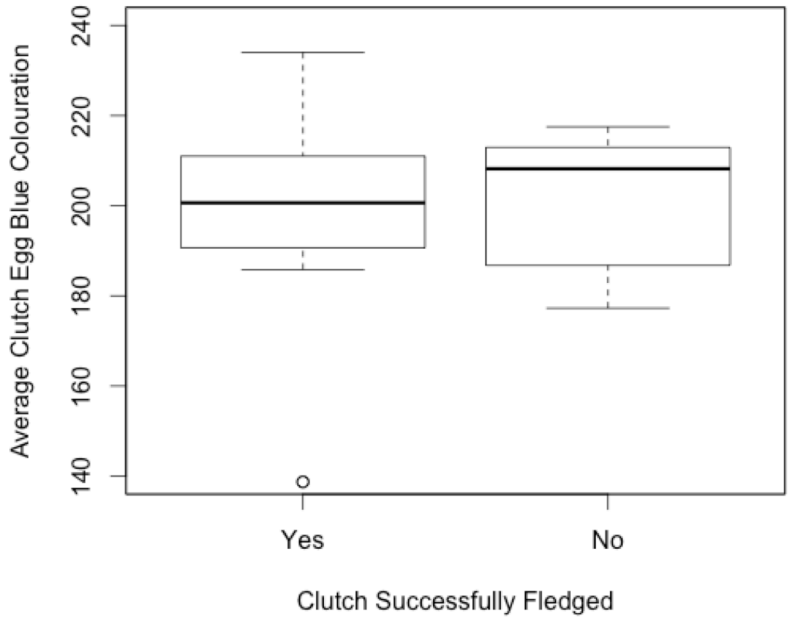

Figure 5. Blue colouration did not differ between clutches that fledged and those that did not.

Discussion

In contrast to previous studies on the adaptive significance of blue egg colouration (Moreno et al. 2004, 2005, 2006a; Soler et al. 2005, 2008; Siefferman et al. 2005; Morales et al. 2006, 2011; English & Montgomerie 2011), I did not find evidence to support the hypothesis that blue egg colouration is acting as a sexually selected signal in the mountain bluebird. Egg colouration was not consistently related to measures of female quality or provisioning rates, (although when a single outlier was removed, I did observe a significant negative relationship between average egg colour and parental provisioning rates.) This null result is consistent with several other studies that have failed to find a relationship between maternal provisioning and egg colouration (Hanley et al. 2008; Walters & Getty 2010; Johnsen et al. 2011; Honza et al. 2011; Stoddard et al. 2012). I also did not find a relationship between egg

colouration and fledging success or nestling growth rates, however this is not surprising given there was no relationship between egg colour and parental investment.

While I did not find evidence to support the sexual selection hypothesis with respect to average egg colouration, I did observe that females that laid clutches with more variation in colour provisioned more than females that laid clutches with less variation in colour. Moreno et al. (2006b) demonstrated a similar relationship in the pied flycatcher, however in their study males provisioned nestlings that hatched from clutches with greater variation in blue colour rather than females. This finding may be related to laying order, as variation in egg colour has been shown to be both positively and negatively associated with laying order (Siefferman et al. 2005; Moreno et al. 2006b). Interestingly, standard deviation in the blue colouration of eggs also appears to be the most heritable aspect of blue egg colouration (Morales et al. 2010a), supporting a genetic basis for within clutch egg colour variation, which selection may be acting upon.

In this study, the most parsimonious explanation for the lack of evidence to support egg colour acting as a signal may be that mountain bluebirds are simply unable to distinguish subtle differences in egg colouration. The inability of males to discern subtle differences in egg colouration under low light conditions (as would they would experience inside a nest box) may mask the effects of egg colouration acting as an honest indicator of female quality. Cavity nesting birds would likely take several minutes to adjust to low ambient light levels in order to visually assess egg colour (Reynolds et al. 2009). Supporting this idea, Cassey et al. (2008, 2012) demonstrated that constraints on avian visual perception may render birds unable to distinguish subtle differences in monochromatic egg colouration, especially in low light environments (e.g. nest cavities). Additionally, because egg colour appears to have a

signaling function in other members of the Thrush family, such as the American robin (English & Montgomerie 2011), blue colouration in mountain bluebird eggs may be the result of phylogenetic constraint rather than adaptive sexual selection. Additional support for this idea comes from phylogenetic analysis showing that blue eggs are least likely to be found in cavity nesting species (i.e. mountain bluebirds) (Kilner 2006).

A possible adaptive explanation for the blue colouration of mountain bluebird eggs is that blue pigmentation serves a structural function There is growing evidence to support the idea that eggshell pigmentation may play an important role in the filtering of harmful UV-B radiation, while allowing wavelengths of UV light that are beneficial to embryo development to penetrate through the shell (Maurer et al. 2015). Structural explanations still leave room for blue egg colouration to act as a sexually selected signal. Blue egg colour may have initially evolved for structural integrity or the filtering of solar radiation but perhaps has been co-opted to function in a signaling capacity in some species (Kilner 2006).

As (Riehl 2011) points out, there may be methodological problems in the testing of the sexual selection hypothesis. The majority of studies have not accounted for correlations between egg colour and nestling traits such as begging posture, nestling size and position, gape colour, sibling competition. Nestling traits have been shown to effect rates of food delivery (Kacelnik et al. 1995; Kilner 1995, 1997, 2002; Saino et al. 2000) and may thus confound the observable effects of egg colouration on provisioning rates. Of all the studies investigating egg colour as a sexually selected signal, only three controlled for nestling traits, and of those studies only two found a significant relationship between egg colour and parental investment (Soler et al. 2008; Moreno et al. 2008; Morales et al. 2010b). Another major criticism of the sexual selection explanation for the blue colouration of bird eggs is

that a vast majority of the evidence in support of the hypothesis comes from a single species, the pied flycatcher (Moreno et al. 2004 p. 200, 2005, 2006a, 2006b, 2013; Morales et al. 2006, 2010a; Johnsen et al. 2011; Avilés et al. 2011). Applying the sexual selection hypothesis as an explanation for the blue colouration of avian eggshells in general presents both practical and theoretical challenges. Although blue egg colour may be sexually selected in some species, it does not work as an adaptive explanation across all taxa, highlighting the importance of studying the evolution of egg colouration across a range of avian taxa with broad variation in life histories.

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APPENDIX A

Table 1A. Results from correlational analysis of the relationship between average nestling growth rates and average clutch egg blue colouration.

	n	*r*	*p*
Average Clutch Weight Day 5	15	0.09	0.74
Average Clutch Wing Day 5	15	0.11	0.67
Average Clutch Tarsus Day 5	15	0.14	0.62
Average Clutch Weight Day 9	19	0.14	0.57
Average Clutch Wing Day 9	19	0.17	0.48
Average Clutch Tarsus Day 9	19	0.06	0.80
Average Clutch Weight Day 13	17	0.12	0.65
Average Clutch Wing Day 13	17	0.15	0.57
Average Clutch Tarsus Day 13	17	0.12	0.66